Environmental Genomics and Carcinogenesis Panels

United States

Chair

Dr. J. Carl Barrett (Chair 1996- , Member 1993-1996)
Director, Division of Basic Research
National Cancer Institute
National Institutes of Health
31 Center Drive
Building 31, Room 3A11
Bethesda, MD 20892

Japan

Chair

Dr. Keiji Wakabayashi (Chair 2000- , Member -1999)
Chief
Cancer Prevention Division
National Cancer Center Research Institute
5-1-1 Tsukiji, Chuo-ku
Tokyo 104-0045, Japan

Panel Members

Dr. Lawrence J. Marnett (2000-)
Director
A. B. Hancock Jr. Memorial Laboratory
for Cancer Research
Department of Biochemistry
Vanderbilt University School of Medicine
Nashville, Tennessee 37832

Dr. Paul S. Meltzer (1997-2002)
Head, Section of Molecular Cytogenetics
Cancer Genetics Branch
National Human Genome Research Institute
National Institutes of Health
Building 49, Room 4A10
49 Convent Drive, MSC 4470
Bethesda, Maryland 20892-4470

Dr. Cheryl Walker (1999-2002)
Associate Professor
Department of Carcinogenesis
University of Texas
M.D. Anderson Cancer Center
Smithville, Texas 78957

Dr. Gerald Wogan (-2000)
Director
Division of Toxicology
Massachusetts Institute of Technology
77 Massachusetts Avenue
Building 16, Room 333
Cambridge, Massachusetts 02139-4307

Dr. Okio Hino (-2000)
Chief
Department of Experimental Pathology
The JFCR Cancer Institute
1-37-1 Kami-Ikebukuro, Toshima-ku
Tokyo 170-8455, Japan

Dr. Nobuyuki Ito (1999)
President
Nagoya City University
1 Kawasumi, Mizuho-cho, Mizujho-ku
Nagoya
Aichi 467-8601, Japan

Dr. Taijiro Matsushima (1977-1996)
Director
Japan Bioassay Laboratory
Japan Industrial Safety and Health Association
2445 Hirasawa, Hadano
Kanagawa 257, Japan

Dr. Masanao Miwa (1994-1998)
Professor
Institute of Basic Medical Sciences
Tsukuba University
1-1-1 Tennodai, Tsukuba
Ibaraki 305, Japan

Dr. Mitsuo Oshimura (　-2000)
Professor
Department of Molecular and Cell Genetics
School of Life Science
Faculty of Medicine
Tottori University
86 Nishimachi, Yonago
Tottori 683-8503, Japan

Dr. Masae Tatematsu
Chief
Division of Oncological Pathology
Aichi Cancer Center Research Institute
Kanokoden 1-1, Chikusa-ku
Nagoya 464-8681, Japan
Dr. Masaaki Terada (1992-1996)
Director
National Cancer Research Institute
5-1-1 Tsukji, Chuo-ku
Tokyo, 104, Japan

Dr. Hiroyuki Tsuda (　2000-)
Chief
Experimental Pathology and Chemotherapy Division
National Cancer Center Research Institute
5-1-1 Tsukiji, Chuo-ku
Tokyo 104-0045, Japan

Dr. Shaw Watanabe (1992-1996)
Chief
Epidemiology Division
National Cancer Research Institute
5-1-1 Tsukiji, Chuo-ku
Tokyo 104, Japan

Guidelines

Environmental Genomics and Carcinogenesis Panels

Experimental Approaches

Explore the multagenic and carcinogenic effects of environmental mutagens and carcinogens, including chemical, physical and biological agents. To study the latest technologies and mechanistic ideas on molecular events which directly or indirectly impact induction of cancer.

1. Incorporate the newest ideas and mechanistic models of transformation of normal cells into tumor cells. This includes regulatory events in cell clycle, both germ cell and somatic cell mutations, signal transduction, DNA repair, oxidative stress, hormonal interactions, mitochondrial pathways, etc.

2. Development and evaluation of transgenic rodent models for assaying mutagenicity and carcinogenicity in short-term and long-term assays.

3. Incorporation of genomic and toxicologic data bases for evaluation of synergistic effects of environmental carcinogens and mutagens.

4. The study of proteins as the effector molecules of gene translation, and the role of post-translational modification on modulation of the cell's regulatory pathways, and the overall effects on disease causation.

5. Utilization of proteomic, mass spectral, and microarray technologies for understanding very early effects of toxic environmental agents.

Studies in Human Populations

Research on the effects of chemical and physical agents in populations based upon the latest molecular knowl-edge of the mechanism of mutagenesis and carcinogenesis.

1. Application of human monitoring methods in subsets of individuals (e.g. waste sites, chemical plants, agri culture, factories, etc.) to determine exposure, molecular binding and disruption of cellular macromolecules by chemicals.

2. Population studies using the role of individual molecular genetic changes for susceptibility to disease by environmental agents. The significance of single nucleotide polymorphisms in metabolism of toxic agents, drug metabolism and efficiency of enzymatic pathways.

3. Incorporation of the latest technologies to monitor work place exposure for the presence of mutagenic or carcinogenic exposures.

Multidisciplinary Approaches

The confluence of human genetic knowledge derived from sequencing the human genome, and the dramatic growth of genetic technologies, has enabled a more rapid and sensitive analysis of the systematic effects to humans by environmental insult.

Five-Year Summary

Broad Goals

The monumental expansion of molecular biology has dramatically altered study of the paradigm of disease causation. Human genetic knowledge derived from advanced biochemical technology has advanced the understanding of the genesis of both genetic and environmentally caused diseases down to the molecular level. Furthermore, these new technologies have enabled analysis of biochemical pathways that maintain cellular homeostasis to come under increasing scrutiny and have led to a better understanding of the validity of the mouse and the rat as surrogate models for human disease. The Environmental Genomics and Carcinogenesis Panels continue to focus on the interaction of genetics and the environment, assembling the burgeoning growth of human and nonhuman toxicological data to increase understanding of their parallel pathways. Thus new ideas can be formulated for further use of both systems in congruence to better understand the molecular bases for mutagenesis and carcinogenesis.

The main thrust of the Panels is to exchange information on all these topics and to foster a collegial atmosphere that is conducive to transfer of information and expertise. Scientists and physicians from many countries are convened in each annual meeting of the Panels. The goal is to disseminate information to laboratories throughout the world, especially those in Asia, developing countries, and areas with high risk of exposure to environmental chemical and physical agents.

Progress and Accomplishments

Research on disease causation has made extraordinary gains in essentially all scientific disciplines during the last 5 years. The ever-enlarging knowledge base has its underpinnings in the rate by which human genomic information has grown. It is anticipated that a readable version of the complete human genome will be available in 2001. Understanding the basic mechanisms of both normal cell growth and transformation to disease is becoming clearer with the deeper knowledge of molecular biology. Mutation that leads to disease can now be better understood at the biochemical level, where a lost or disrupted step in the normal regulatory processes of the cell can be more directly located. The Panel meetings during the last 5 years have clearly exhibited the explosion in technology, including the most current scientific areas of research, such as endocrine disruptors, oxidative stress, transgenic models, proteomics, microarray technology, and single nucleotide polymorphisms.

Cancer is a heritable disorder of somatic cells, and both environment and heredity operate simultaneously in its origin. One of the most critical areas of research is the possible relationship of cancer to toxic environmental chemicals that appear to have estrogenic activity and their effect on reproductive failure and cancer. Uterine leiomyomas (fibroid tumors) are the most common gynecologic malignant tumor of the female reproductive tract. Study findings suggest that the process of carcinogenesis is dependent on ovarian steroid hormones and that the action of environmental compounds on the endocrine system may contribute to development of these tumors. Also, known risk factors for breast cancer explain approximately 30% of the variability in incidence. Risk factors for the remaining 70% are unknown, partly because breast cancer is very heterogeneous, with multiple factors contributing to the etiology of the disease.

Malignant transformation may be caused by a number of lifetime events and exposures, in combination with variability of key genes that metabolize steroid hormones, dietary factors, and chemical carcinogens, as well as those involved with DNA repair, signal transduction, and cell-cycle control. Identification of subgroups of women who are very susceptible to particular agents, as evidenced by variability in response, can more clearly reveal associations between disease risk and factors that were previously unclear. The underlying mechanism of tumor promotion by these compounds appears to result from an increased responsiveness of tumor cells to signaling via the estrogen receptor. Tumor cells display an increased capacity to proliferate at low hormone levels and a decreased apoptotic response to hormonal withdrawal.

Chlorinated dioxins are an example of this large family of ubiquitous environmental contaminants that produce a spectrum of adverse biochemical and biological effects, including reproductive, developmental, and carcinogenic effects in humans and in a wide variety of experimental animal models. The general scientific consensus is that most if not all effects of these compounds are mediated by initial binding to the arylhydrocarbon receptor (AhR). For example, the compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) promotes liver tumors in female Sprague-Dawley rats.

However, hepatocarcinogenesis and proliferative action of TCDD are observed only if the ovary is intact. This finding suggests that the tumor-promoting effect of TCDD may be mediated either by direct modulation of ovarian function or through endocrine regulation.

It is becoming increasingly clear that individual differences in specific genes can have profound effects on the ability to detoxify chemical agents, as well as the degree of receptivity to certain medicines. These slight variations in gene sequence, single nucleotide polymorphisms (SNPs), can have a marked effect on the structure of the translated protein, which then can affect its ability to react with its natural substrate. SNPs are the most common form of variation and disease-causing mutations in the human genome. Because these variants occur frequently, they are likely to be associated with most phenotypic differences in humans. Emerging results suggest that the level and patterns of sequence variation found in human genes could pose a challenge to identification of the sites or combination of sites that influence variation in risk of disease within and among human populations. For example, polymorphism of the CYP2A6 gene is responsible for the activation of promutagens and carcinogens in tobacco. Gene deletions have been seen in subpopulations of persons who have poor metabolism, and these persons may have a reduced risk of lung cancer because metabolic activation of these carcinogens is lacking.

The repair of DNA damage is critical to protecting cells from cancer-causing agents. Recent reports suggest that persons with even marginal reductions in the capacity to repair DNA damage have increased susceptibility to breast, lung, and skin cancer. One study of repair genes showed numerous SNPs, many resulting in amino acid substitutions. In addition, biochemical analysis has shown that many of the amino acid substitution variants are associated with reduced function, which is probably related to relative susceptibility to disease. Results from genotyping of variants in cancer patients and control subjects indicate that several variant alleles may be associated with cancer risk.

Understanding of this complex interaction of genetics and environmental agents will require collection and processing of qualitative and quantitative data on many genes and numerous enzymatic pathways that are affected by estrogen-like agents. In recent years, gene-chip technology has allowed the assay of thousands of genes at one time, and this method has become increasingly important for tracking the process of mutagenesis and carcinogenesis. Expression profiling, by use of large-scale monitoring of gene expression to investigate cellular mechanisms of toxicity, will soon be widely used. Microarrays are manufactured to represent entire cellular processes, including responses of the immune system, receptor biology, signal transduction, protein modification, membrane transport, growth and development, chromatin metabolism, cell adhesion and kinesis, and regulation of the cell cytoskeleton. For example, by using microarrays of several hundred genes with relevance to mechanisms of toxicity, one can examine human breast and uterine cell lines toward natural and environmental estrogens and antiestrogens, to identify transcriptional targets of subtypes of the estrogen receptor.

Genes drive the biological functions that characterize and distinguish tissue types. Until recently, it has not been possible to simultaneously and quantitatively measure the expression levels of thousands of human genes that characterize a tissue. By developing large gene arrays to determine the whole range of normal gene function, it will be possible to rapidly screen for suspected abnormal conditions seen in disease. With use of this process, specific tissues can be assayed for unique sensitivity to suspected environmental toxicants.

The meteoric progress in sequencing the human genome has resulted in a concomitant surge in proteomics: the study of the identity and function of all families of proteins expressed by the genome at any given time or condition. It is generally recognized that proteins are the defining elements for understanding cell structure and function. This knowledge will be critical for understanding the underlying mechanisms of disease causation. Because proteins, the effector molecules, consist of 20 building blocks, as opposed to nucleic acids, which are built from four, the degree of structural complexity manifested in protein is far greater than that in nucleic acids. Primary protein structure deduced from genomic templates does not yield an accurate map of the final active species of a protein. Nascent proteins detaching from ribosomes form straight chains and must be modified post-translationally by removal of amino acids, addition of numerous other side-chain molecules, or both, all of which contribute to solubility, as well as the secondary and tertiary structure of the molecule. All these post-translational changes are critical to the ultimate functionality of the protein, because they determine how the protein is to be folded in its final structural transformation into a unique three-dimensional shape. The shape of each protein species is unique, so each protein will usually catalyze only one chemical reaction, and only a single ligand can fit into its active site. The myriad of biochemical pathways function and interact with each other. As this knowledge grows for the biochemistry of both normal and abnormal conditions, the chronology, the control steps for each pathway, and the molecular reasons for mutational errors that lead to disease will become apparent.

For more complete characterization, a protein is digested into peptides and analyzed by tandem mass spectroscopy (MS)-quadrapole MS, which is used to determine the correct amino acid sequence of individual proteolytically prepared peptides, for comparison in a database for exact identity. This process will show amino acid substitutions, as well as post-translational modification. In addition, it has become apparent that SNPs have a critical role in an individual's susceptibility to disease. A genetic polymorphism may have several separate effects on a protein. It can result in no coding change of an amino acid, or it can cause an amino acid change in a region of the protein that does not perturb the active site or the conformational shape necessary for a proper fit of the ligand. Both types of changes are "silent" SNPs. However, the SNP may occur in a region of the protein that will modify its ability to react with a ligand, either by inhibiting function or by altering its reaction kinetics sufficiently to hamper normal enzymatic activity. It is estimated that there are approximately 17 million SNPs in the human genome, with about 5%, or 500,000, expected to be in coding regions (average, about six SNPs per gene). Of course, that calculation does not take into account the potential synergistic effect of SNPs in proteins involved in post-translational modification.

Future Goals

The growth of genomic knowledge and molecular biological techniques will continue at an ever-increasing rate. Sharing results, ideas, and new hypotheses will be more and more important for the Panels. Historically, both the United States and Japan have produced frontier research in mutagenesis and carcinogenesis. Perusal of past activities shows the evolution of the Panels' direction in concert with the new and exciting approaches to disease research. In the early days, the emphasis was on whole-animal studies and short-term mutagenesis assays. In the last several annual meetings of the Panels, there has been increased emphasis on (1) the growth in knowledge about the mechanisms of mutagenesis and carcinogenesis and (2) the development of transgenic rodent models.

It is anticipated that the next 5 years will see active collaboration and discussion on the newest research concepts in mutagenesis and carcinogenesis, which span basic research in both human and model systems, as well as epidemiologic studies focused on the impact of environmentally toxic agents.

Selected References

United States

Barrett JC. Molecular and environmental causes of cancer. *Drug Metab Rev* 2000;32:139-42.

Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis* 2000;21:427-33.

Hogdges LC, Bergerson JS, Hunter DS, Walker CL. Estrogenic effects of organochlorine pesticides on uterine leiomyoma cells in vitro. *Toxicol Sci* 2000;54:355-64.

Marnett LJ, Wright TL, Crews BC, Tannenbaum SR, Morrow JD. Regulating of prostaglandin biosynthesis by nitric oxide is revealed by targeted deletion of inducible nitric oxide synthetase. *J Biol Chem* 2000;275:13427-30.

Spalding JW, French JE, Stasiewicz S, Furedi-Machacek M, Conner F, Tice RR, Tennant RW. Responses of transgenic mouse lines p53(+/-) and Tg.Ac to agents tested in conventional carcinogenicity bioassays. *Toxicol Sci* 2000;53:213-23.

Japan

Daigo Y, Nishiwaki T, Kawasoe T, Tamari M, Tsuchiya E, Nakamura Y. Molecular cloning of a candidate tumor-suppressor gene, DLC1, from chromosome 3p21.3. *Cancer Res* 1999;59:1966-72.

Kitabayashi I, Yokoyama A, Shimizu K, Ohki M. Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. *EMBO J* 1998;17:2994-3004.

Miyamoto M, Umetsu Y, Dosaka-Akita H, Sawamura Y, Yokota J, Kunitoh H, Nemoto N, Sato K, Ariyoshi N, Kamataki T. CYP2A6 gene deletion reduces susceptibility to lung cancer. *Biochem Biophys Res Commun* 1999;261:658-60.

Nukaya H, Yamashita J, Tsuji K, Terao Y, Ohe T, Sawanishi H, Katsuhara T, Kiyokawa K, Tezuka M, Oguri A, Sugimura T, Wakabayashi K. Isolation and chemical-structual determination of a novel aromatic amine mutagen in water from the Nishitakase River in Kyoto. *Chem Res Toxicol* 1997;10:1061-6.

Ochiya T, Takahama Y, Nagahara S, Sumita Y, Hisada A, Itoh H, Nagai Y, Terada M. New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet. *Nat Med* 1999;5:707-10.